The Bacterial Genus *Lineola*

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SUMMARY: *Lineola*, a new bacterial genus, is characterized by large and very long cells of an unusual type and by the formation of motile trichomes of considerable length which may be branched.

Two species were isolated in pure culture by the use of capillary pipettes. Even when their nutritional requirements were known it was difficult to purify them by plating. The growths of the two species on agar are different because the trichomes of *L. articulata* are much softer than those of *L. longa*.

The cells of both species contain a large number of nucleoids and divide by constriction. In *L. longa* the daughter cells remain firmly connected, whereas in *L. articulata* the links become pliable, the whole chain-like trichome bending when swimming. Both species are Gram-negative and have numerous flagella. No spores were found.

Media with peptone, meat extract, yeast extract, and the like must be supplemented with acetate and with agar or certain other substances, to support multiplication.

In a previous paper (Pringsheim & Robinow, 1947) mention was made of a bacterium which was provisionally named *Lineola longa* because O. F. Müller had observed a *Vibrio lineola* possibly identical with the newly isolated form. From Müller's figures (1786, pl. VI) it was clear that his organism was not the same. Since no indication could be found that *Lineola longa* had previously been described, and as the generic name *Lineola* has never been used, this species is now proposed as the type of a new genus.

In addition, a second species was isolated for which the name *L. articulata* is proposed. Other, smaller, species exist which can probably be included in this genus. One with slightly pointed ends and only 1.0–1.2 μ . wide was recognizable. It is, however, the great size of the cells which gives interest to the two species described here. They may, like *Caryophanon* (Peshkoff, 1940; Pringsheim & Robinow, 1947), be useful for investigations on cell-physiology, osmotic behaviour, and movement of bacteria. Cultures for investigations will be supplied on request and will be deposited with the American Type Culture Collection.

The two species of *Lineola* are found in decaying plant material from quiet waters, *L. longa* also in infusions of fresh cow-dung. It is surprising that they have never attracted attention, but they are not mentioned in Migula's *System der Bakterien* (1900) nor in *Bergey's Manual* (1939, 1948). This omission is probably due to the difficulty in obtaining pure cultures of these organisms by ordinary bacteriological methods.

Isolation

L. longa, although observed previously on several occasions, was first recognized in infusions of cow-dung from Cambridge in October 1945. It attracted attention by the movements of its trichomes. These are sufficiently

large to be sucked into capillary pipettes under a dissecting microscope and freed from contaminants by three transfers to sterile washing fluids (Pringsheim, 1937, 1946). The first pure culture was obtained in cow-dung extract supplemented with 0.2 % meat extract. When its requirements were better understood it was possible to obtain isolated colonies of another strain on agar plates by streaking out from a dense population in polluted water. After 2 days at room temperature the large colonies could be recognized and were sufficiently distinct from other growths to be isolated with the help of a capillary pipette.

L. articulata was detected in September 1947 under similar circumstances in an infusion of decaying plant material from the New Forest. My attention was drawn to it by the flexibility of the long chains of rods as they swam through the water. This species was also seen several times subsequently in similar micro-communities. Isolation was performed in much the same way as with *L. longa*. Purification by plating was not successful. Its requirements for growth are similar to those of the other species, except that it develops more readily in liquid media and is not so liable to lose its motility.

Cultural appearances

Both species of *Lineola* form chains of rods of such width that the growths can be recognized as filamentous with a strong hand lens. Colonies on agar are, however, different and characteristic in the two species. It is several days before single trichomes attain sufficient size to become visible to the naked eye.

Large colonies of *L. longa* on agar are flat, irregularly shaped semi-confluent patches with fringes at the edges and are composed of long trichomes (Fig. 1*a, b*) as in other filamentous bacteria (Bisset, 1939). Single organisms give rise to small medusa-head colonies. When they have grown larger they are bluish white when viewed against a dark background and show a watered silk effect. The organisms cohere so that they must be removed almost completely as a colony for transfer to a fresh medium. If the colony is only touched with the needle, nothing may adhere, and subcultures are sterile. The appearance of the growth is evidently influenced mainly by the length and rigidity of the trichomes.

L. articulata does not produce fringed colonies because of the flexibility of the filaments which are kept together by the surface tension of the water-film covering the colonies on agar. Large colonies are wavy or lobed in outline and curly in structure (Fig. 2). They are more like mother-of-pearl than watered silk, and are iridescent rather than bluish. *L. articulata* is less coherent than *L. longa* and more readily transferable.

The youngest growths of the two species on agar are as different as are the older ones. Viewed by low power 1 day after inoculation *L. longa* appears as more or less parallel and straight threads. By elongating and breaking up into shorter lengths the trichomes soon form bundles (Fig. 3*a*). Isolated trichomes form, by intercalary elongation, coiled aggregations at intervals, connected by almost straight sections (Fig. 3*b*). Still later a maze of curved filaments is formed so that the initial arrangement can no longer be recognized.

Young growths of *L. articulata* appear as irregularly undulating snake-like threads which develop into small, narrow elongated colonies. These later

become V-, Y-, or X-shaped and develop into increasingly regular patches, at first still showing points and outgrowths which, however, change later into spiral areas within the large colonies (Fig. 4*a, b*).

Fig. 1*a, b*. Colonies of *L. longa* on acetate peptone yeast extract agar; (a) $\times 25$; (b) detail. $\times 50$.

Fig. 2. Colonies of *L. articulata* on acetate peptone yeast extract agar. $\times 25$.

Whereas both species grow abundantly in the condensation fluid of agar slopes, their multiplication in liquid media occurs only in the presence of certain combinations of substances. At first multiplication was obtained only when cow-dung extract was added to a medium on which growth could otherwise be secured only in combination with agar. Later cow-dung was found to be replaceable by soil extract or dilute sea water, though not quite so effectively with *L. longa* as with *L. articulata*. The addition of very small amounts of agar (0.05–0.2 %) proved also to be beneficial. Even distilled water in which agar had

been soaked, added to a medium which without agar did not support growth, permitted the development of both species of *Lineola*.

In all liquid media growth of *L. longa* in a non-motile state is best near the surface, although when slight or in the early stages, it may start near the bottom. If it is not too meagre a ring is formed just below the meniscus,

Fig. 3a,b. Young colonies of *L. longa*; (a) after 1 day: trichomes still straight; (b) after 2 days: elongating filaments curling up. $\times 25$.

Fig. 4a,b. Young colonies of *L. articulata*; (a) after 1 day: snake-shaped owing to softness of trichomes; (b) after 2 days: trichomes sliding along one another during elongation and, when frictional resistance increases, curling in the middle of growing colonies. $\times 25$.

which becomes detached and falls to the bottom on the slightest disturbance. When growth is rapid and motility strong, *L. longa* produces a nearly homogeneous turbidity. In less favourable conditions motility is diminished and the trichomes are twisted and entangled, so that their aggregates can be seen with the naked eye as silky white floccules. Sometimes filaments of enormous length develop, eventually forming deposits like cotton-wool on the bottom of the tube.

L. articulata, which is rarely non-motile, fills the whole fluid with evenly distributed trichomes. These are seen with a hand lens to swim in all directions at random, never aggregating and only settling on the bottom in ageing cultures.

In agar stab cultures growth of both species develops only on or very near the surface. This is true even with not more than 0.25 % agar which suffices to prevent the inoculum from falling to the bottom or moving about.

Morphology and movement

Lineola longa

Under natural conditions and in favourable media *L. longa* appears in the form of long, slightly and irregularly curved trichomes, 1.4–1.6 μ . in diameter and of great length. Free-swimming trichomes may be more than 200 μ . long; the longer they are, the less regular and lively is the movement. Even the longest ones are relatively rigid, although a slight pliability and elasticity can be discerned when the trichomes encounter mechanical obstruction.

Fig. 5. Growing motile trichomes of *L. longa* after fixation with osmic acid: division by constriction and fission. $\times 750$.

All but the shortest trichomes are subdivided by constrictions which accentuate the demarcations between the rod-like sections but do not at first truly divide the interior of the cells. Later they are completed to become cross-walls (Fig. 5). These can be demonstrated with tannic acid-crystal violet (Pringsheim & Robinow, 1947, p. 276 and pl. 5, fig. 16). By Giemsa-staining it can be shown that these rod-shaped sections are not further subdivided; they thus form cells, 25–40 μ . long, only a relatively small number of which constitute even the longest trichomes. As each rod-shaped section contains a considerable number of Feulgen-positive bodies, presumably of a nuclear nature, the sections are comparable to multinucleate cells or coenocysts of algae.

Young trichomes, 50–60 μ . long, begin first to show a slight constriction at about the centre. As the whole trichome grows longer the constrictions become narrower, while new ones appear in the two halves, though generally not at regular intervals. The majority of trichomes in a healthily growing culture are composed of four incompletely separated sections; when the first constriction is completed by the formation of a cross-wall the filament divides into two by transverse fission at this place.

Strangely enough the trichomes are sometimes branched, this phenomenon being quite frequent at times. Sometimes branched forms are composed of only three rods. When the main trichome was considerably longer than the side-branch the movement was almost normal, in which case the branch rotated with the entire structure. More fully developed branches rendered the movement irregular, so that progress became almost impossible. The cells were plasmolysed in the normal way in hypertonic solutions (cf. p. 206).

Fig. 6. *L. articulata* from agar slope; flagellar staining, preparation by Dr W. J. Dowson. $\times 2000$.

L. longa stains like other bacteria. It is Gram-negative and when in a healthy state does not contain appreciable amounts of granular material; these, however, accumulate in old cultures. It is motile by a large number of peritrichously arranged flagella. They are evenly distributed over the whole of the trichomes. No mucilage or sheath could be demonstrated. When a filament is torn, the remainder of the longitudinal cell-walls have the appearance of a sheath, but on closer inspection their true nature is seen. The bacterium is fairly constant in size, shape and reaction to cultural conditions. No difference between various types of colonies, e.g. roughness and smoothness, was observed.

Lineola articulata

This species resembles *L. longa* in many features. The cells are $1.4\text{--}1.6\mu$. wide and $30\text{--}50\mu$. long. They stain and are covered with flagella much in the same manner as *L. longa* (Fig. 6). The elongation, division by constriction and subsequent separation of the cells are also the same.

The distinctive character is the pliability of the connecting links, once the cross-walls separating the cells have been completed. How the cells remain attached is not clear. Narrow gaps may be seen between them, so that they are apparently unconnected, although linked up to form tough coherent trichomes (Fig. 7) which never break during movement, not even when a trichome is sucked into a capillary and blown out again. The joints must therefore have a remarkable toughness, in spite of being much more pliable than the inflexible cells. At the same time they possess a certain degree of rigidity since, during forward movement, the same slight curvatures often reappear again and again after each rotation (Fig. 8*d*).

Fig. 7. *L. articulata*, cells with granules, trichome with constrictions, and branching. $\times 2000$.

No material filling the apparent gaps nor any sheath bridging them could be detected by staining with methylene blue, safranin or congo-red, nor by means of India ink and nigrosin, in living or in dried preparations. Dark-ground illumination was similarly unsuccessful. It is difficult to imagine any substance tough enough to hold the sections together and yet so watery that India ink and nigrosin could penetrate. No evidence of linkage by means of delicate threads or plasmodesmata could be found.

Branching is more frequent in *L. articulata* than in *L. longa*. Side branches are attached to the main trichome in the same indefinable manner as the sections of the original trichome, i.e. seemingly unconnected and yet held in their places in spite of vigorous movement.

Free-swimming trichomes consist generally of a greater number of cells than those of *L. longa*. Often sixteen of them are found linked together, but irregularities in cell-division occur, so that the number, after four divisions, is usually less. In any population there are short trichomes also, consisting of two or more cells which eventually grow into long ones.

When undisturbed most of the filaments swim over a limited distance in a nearly straight line, so that it is clear that the flagella are well synchronized. A few trichomes, and many more after agitation, assume a more or less curved shape. They may swim in a circular course, curling up to form spirals, or they become horse-shoe shaped, progressing for a little while with the convexity in front or continually changing their direction, until one of the arms prevails and pulls the rest along so that the whole trichome becomes straight again. Simple filaments move and curve gracefully in a manner reminiscent of railway trains in a marshalling yard (Fig. 8*a-c*).

Fig. 8

Fig. 9

Fig. 8. *L. articulata*, schematic sketches of moving trichomes. (a) 1-6: stages in the movement of a trichome; (b) 1-4: the same, depicting trichome with one unicellular branch attached; (c) motile trichome with two multicellular branches; (d) two stages of a rotating trichome with characteristic curvature, given as if it had not moved forward meanwhile, in order to show the symmetry of the two appearances. The unaltered shape indicates a marked rigidity in spite of the pliability often exhibited. $\times 50$.

Fig. 9a,b. *L. articulata*: plasmolysis; (a) artificial sea water of double concentration; (b) 5% glucose solution.

Fig. 10a-c. *L. longa* branched trichome. (a) two cells attached to a joint; (b) four cells near to one joint; (c) complex aggregation of cells. (a) and (b), $\times 2000$; (c) $\times 400$.

From the appearance and variation of these movements it is clear that they are caused by the action of the flagella. The individual cells do not change shape while rotating. This is best seen when there are slight irregularities in their form. When the larger part of a trichome drags along a portion bent in another direction obviously struggling to have its own way, one can watch the action of the conflicting forces and their influence in decreasing the speed and regularity of progress.

An interesting comparison is provided by *Bactoscilla flexibilis* (Pringsheim, 1949, p. 72), belonging to the Vitreoscillaceae, which are characterized by their gliding movements, combined with bacterium-like shape and size of cells. Its rod-shaped sections are linked together, so that during movement the trichome bends at the joints between the rods. The connecting matter is invisible, and the rods themselves remain straight. The movement, however, unlike that of *Lineola articulata*, is not caused by flagella; the trichomes adhere, at least partly, to the substrate while they glide along, and the joints appear to bend actively, not passively.

A further significant difference between the organization of *Lineola* and that of the gliding organisms is the osmotic behaviour of the cells. Whereas in Myxophyceae, Beggiatoaceae and Vitreoscillaceae (Pringsheim, 1949) the cells shrink as a whole in hypertonic solutions, plasmolysis can be beautifully demonstrated in *L. longa* and in *L. articulata* (cf. p. 203). In sea water, for instance, and in glucose solutions the cytoplasm retreats from the cell-wall in many places leaving more or less hemispherical cavities filled with the plasmolysing fluid (Fig. 9*a, b*). The cell-walls remain in about the same position and are clearly seen, while nothing of the kind could be detected in similarly treated trichomes of *Vitreoscilla moniliformis*, the largest of the species of this colourless gliding genus.

Branching

Branching occurs in both species, more frequently in *Lineola articulata* than in *L. longa*. The incessant movement made it impossible to investigate the origin of the branches. Their development could only be interpreted on the ground of their appearance. The use of fixatives and stains was ineffective. On application of osmic acid vapour, formalin and mercuric chloride the trichomes are disrupted into single cells or pairs. Direct addition of osmic acid solution caused the movement to cease while many trichomes were still coherent, but a real attachment becomes obvious only when the trichomes are in motion. Various vital stains were tried without useful results.

Most branches consist of single rods or short chains but, especially in *L. articulata*, longer side branches are not uncommon so that it was sometimes impossible to tell which was the main trichome and which the branch. In *L. longa* two, three or even more cells may be attached to the main axis near to one another (Fig. 10), while in *L. articulata* more than two were not found at one joint.

The branches are almost always attached where the cells meet. Only when more than two are close together, the odd ones are shifted slightly to the side. As the cells divide by transverse fission and not longitudinally, the

phenomenon of branching is difficult to explain. The habitual insertion of side branches at the joints is scarcely compatible with 'false branching' as it is found in Myxophyceae and in *Cladothrix*. No stages were found which could be interpreted this way.

There remains the possibility that previously independent cells settle on other trichomes. This interpretation cannot readily be accepted because these bacteria are never found attached to other bodies, but certain observations are in its favour. The predilection for the joints would, of course, require to be explained by special properties they possess. Chemotaxis is not probable. It is, however, at least feasible that the connexion between the rods is effected by some adhesive substance to which other cells may also stick, although in rare instances cells and cell-chains were found attached near the middle of a cell, so that it would be necessary to assume that the cell ends retain their adhesive nature after separation. This is not very probable since they do not stick to other bodies. Moreover, particles of India ink, although sometimes adhering to the organisms, did so completely at random without any preference for the ends or joints. The problem therefore remained unsolved. The actual process of attachment also could not be watched; but two or three cases were observed where a side-branch became detached.

Comparison with other bacteria

The organization of *Lineola*, characterized by the very long multinucleate cells, the contractions which mark far in advance the places of division, and the formation of consistently motile trichomes, may in the first instance appear curiously different from that of other bacteria, just as in another way do those of *Caryophanon* (Peshkoff, 1940; Pringsheim & Robinow, 1947) and *Oscillospira* (Pringsheim, 1949, pp. 72, 99). It is therefore necessary to discuss the differences and similarities between *Lineola* and other bacteria.

To begin with the largest forms, the shape of the cells of *Caryophanon* and *Oscillospira* is of course very different, these two genera having short discoid cells. Similarity is, however, found in the early accentuation by constriction of the places where separation will later take place. A difference is again found in the sections of *Caryophanon* and *Oscillospira* between two constrictions being composed of a number of serially arranged cells, whereas those of *Lineola* are unicellular, cross-walls not being formed between the nucleoid structures. Similar differences are found in certain groups of algae, for example, the Chlorophyceae, where the species *Ulothrix* and *Cladophora* represent organizations somewhat parallel to *Caryophanon* and *Lineola* respectively.

As long as the rods of well-known bacteria were mistaken for cells, the difference between them and *Caryophanon* was considerable, but since we know that they are in most cases composed of several cells (Robinow, 1945; Pringsheim & Robinow, 1947, p. 272, fig. 1), the division of their rods is comparable with the constriction and separation of the sections of *Caryophanon* and *Lineola*. The latter differs mainly in the lack of septa dividing the multinucleate sections into cells. Except for the nucleoids, *Bacillus anthracis* resembles *Lineola* in its cylindrical cells (Flewitt, 1948, pl. 1, fig. 4), whereas

Corynebacterium is more like *Caryophanon* in its shorter cells and their greater number in one rod (Bisset, 1949, p. 94, figs. 1, 2). Common to all these bacteria is the constriction, not immediately followed by division.

Nutrition

A medium containing 0.3 % yeast extract, 0.1 % meat extract, 0.1 % sodium acetate and 1.5 % agar was recommended in the previous paper as suitable for *Lineola longa*. No analysis of the exact nutritional requirements has yet been undertaken but, as far as is known, there are in this respect hardly any differences between the two species *longa* and *articulata*. A remarkable feature is the favourable effect of acetate, already found for *Caryophanon latum* which, however, can multiply in the absence of fatty acids, though neither species of *Lineola* can do so. The latter require even higher concentrations of acetate in order to thrive than are favourable for *Caryophanon*. When *L. longa* is transferred to the surface of an otherwise suitable agar medium but without acetate, only a very slight elongation of the trichomes takes place. When 0.1 % sodium acetate is added to the medium, the short trichomes grow out into long threads which, by intercalary elongation, are transformed into spirals (cf. p. 199).

Both species grow on agar media with acetate and meat extract or yeast extract, but much more profusely when peptone is added. An agar medium containing 0.2 % sodium acetate, 0.2 % yeast extract (Difco), and 0.2 % Bactotryptone with 1–1.5 % agar affords better growth than that given above. Strict neutrality as required by *Caryophanon* and other bacteria is not necessary. When the concentration of the nutrients is doubled no more multiplication occurs.

Neither glucose nor glycerol have any beneficial effect. Larger amounts of mineral salts, for example sea water, even diluted down to 1/4, are noxious. Growth is not appreciably quicker at 25–27° than at 18–20°. At 32° growth of *Lineola longa* is retarded, but *L. articulata* is still able to grow.

The results so far reported were obtained with agar-slope cultures. Liquid media proved more difficult to use, although both species are found in aqueous habitats. Dung extract and soil extract were suitable supplements to liquid media containing acetate, yeast extract and tryptone, which themselves could not support growth. Sea water diluted to 1/10 and Benecke solution were partially effective but not reliable, and agar in low concentrations was beneficial. *L. articulata* reacts more readily than *L. longa* to such additions. None of the growth factors contained in meat extract, yeast extract, liver extract, nor aneurin, can replace dung extract, so that the real nature of the relevant factor remains unknown.

DIAGNOSIS

Lineola Pringsheim, n.g.

Characterized by very long peritrichous rods, composed of one or two multinucleate cylindrical cells; non-sporing; Gram-negative. Forms motile often branched trichomes up to several hundred microns in length. The rods do not break up into smaller units at the end of the life cycle but divide into two by constriction and fission. Type species *L. longa*.

***Lineola longa* n.sp.**

Cells 1.4–1.6 μ . wide and 10–50 μ ., mostly 25–40 μ . long. Trichomes rigid, up to 800 μ . long. Non-motile trichomes of much greater length may be formed. Grows in pure culture on agar with yeast extract or meat extract, peptone, and acetate; in liquid media only when supplemented with extracts of dung or soil, or other growth-promoting substances. The type culture is that obtained from infusion of cow-dung from Cambridge on 25 October 1945. The subculture has been deposited with the American Type Culture Collection.

***Lineola articulata* n.sp.**

Cells 1.4–1.6 μ . wide and 10–50 μ ., mostly 30–40 μ . long. Motile trichomes up to 160 μ . in length with flexible joints connecting the individual rods.

Type culture obtained from water with plant debris from the New Forest, 80 September 1947. Nutrition similar to that of *L. longa*. Subculture deposited with the American Type Culture Collection.

Both species are frequently found in nature where plant material is decaying.

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